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Role of Interleukin 1 in Antigen-Induced Exacerbations of Murine Arthritis

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The mechanism underlying the chronic and intermittent course of rheumatoid arthritis is not elucidated. In the present study, the role of interleukin 1 (IL-1) was investigated in exacerbations of antigen-induced arthritis in mice. A flare-up of smoldering inflammation (weeks 3 to 4 of antigen-induced arthritis) was inducible by injection of a small amount of methylated bovine serum albumin into the hypersensitive knee joint. Immunohistochemistry showed IL-1 expression in the synovial lining layer and in focal areas of the inflamed synovium during the flare-up. IL-1 was also measured in 1-hour culture supernatant of synovial tissue taken during the flare-up by a bioassay. The expression of both immunoreactive and bioactive IL-1 in the hypersensitive joint peaked around 6 hours after antigen (2 µg of methylated bovine serum albumin) injection and declined thereafter. Antigen rechallenge induced an acute joint swelling of the arthritic joint but not in the naive joint of the sensitized mouse, yet synovia of both joints produced IL-1 after antigen injection. Remarkably, a single intravenous injection of rabbit anti-IL-1 α and -β antibodies 1 hour before antigen rechallenge neutralized IL-1 in the joint. Anti-IL-1 treatment significantly reduced the antigen-induced joint swelling (30 to 40%) but did not affect the profound influx of polymorphonuclear cells in the onset of the exacerbation. However, a profound relief of the inflammation (synovitis) was obtained by IL-1 blockade on day 4 of the exacerbation. Chondrocyte proteoglycan synthesis was markedly suppressed in the antigen-challenged naive knee joints suggesting that this was a direct IL-1 effect as the inflammation was insignificant. Anti-IL-1 treatment was able to maintain chondrocyte proteoglycan synthesis in the antigen-rechallenged joint, which

was highly suppressed in the control group. Furthermore, the enhanced proteoglycan breakdown in the antigen-rechallenged joints was significantly decreased in the anti-IL-1 group. We concluded that IL-1 is an important mediator in exacerbations of murine arthritis, and amelioration of cartilage pathology was obtained with anti-IL-1 antibody treatment. (Am J Pathol 1995, 146:239-249)

There is increasing evidence to suggest that the pluripotent cytokine interleukin-1 (IL-1) plays an important role in the pathogenesis of inflammatory joint diseases, including rheumatoid arthritis. IL-1 has been identified in the synovial membrane and cartilage-pannus junction of arthritic joints from patients with rheumatoid arthritis.^{1,2} Moreover, numerous studies reported a striking correlation between the IL-1 levels in rheumatoid synovial fluid or plasma and the disease activity in these patients.³⁻⁵ The two important features of arthritis, namely inflammation and cartilage destruction, can be attributed to local IL-1 activity. In several mammalian species it was demonstrated that IL-1 injected into the synovial joints causes an arthritic insult.⁶⁻⁸ *In vitro* studies showed that chondrocyte proteoglycan (PG) synthesis was more susceptible to IL-1 than chondrocyte-mediated PG degradation.⁹⁻¹¹

To prove the importance of IL-1, recent studies have addressed the effect of blocking IL-1 in experimental arthritis either by neutralizing IL-1 with antibodies or soluble IL-1 receptors or by antagonizing the IL-1 binding to its receptor with IL-1 receptor antagonist protein (IL-1ra) and related proteins (M20). These different therapeutic approaches were able to diminish joint inflammation and also to reduce cartilage destruction in collagen-induced arthritis (CIA), immune complex arthritis (ICA), and adjuvant arthritis

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and in the autoimmune prone MRL/lpr mice.¹²⁻¹⁷ In murine antigen-induced arthritis (AIA), anti-IL-1 treatment did not affect the acute joint inflammation (edema and influx of polymorphonuclear cells; PMNs) nor the accelerated PG breakdown, yet the marked chondrocyte PG synthesis inhibition was completely prevented.¹⁸⁻²⁰ We were the first to demonstrate impressive reduction both in inflammation as well as in cartilage destruction in established CIA using rabbit anti-IL-1 polyclonal antibody treatment.¹³ These studies at least suggest that IL-1 blockade is worth pursuing as a therapeutic strategy for human arthritis.

In 70% of patients with rheumatoid arthritis, the disease is characterized by an intermittent course of inflammation.²¹ In humans, a rise in plasma IL-1 β levels followed the secondary flare-ups in RA, and isolated peripheral monocytes spontaneously produced IL-1 during the flare-up.^{3,22} In the present study, experiments were carried out to examine the role of IL-1 in the flare-up with an animal model. A flare-up of smoldering inflammation in the chronic phase of AIA was inducible in a predictable and synchronized manner by injection of a small amount of methylated bovine serum albumin (mBSA) into the hypersensitive knee joint.

Recently, Schwab et al²³ demonstrated that IL-1 played a key role in the peptidoglycan-polysaccharide polymer reactivation of streptococcal cell wall-induced arthritis. It cannot be excluded that at least part of the flare-up reaction in this model was nonimmunologically mediated.²⁴ Apart from peptidoglycan-polysaccharide, flare-ups can also be induced with cell wall-derived lipopolysaccharide and these fragments can directly activate macrophages. In AIA, T lymphocytes, as part of the residual inflammation in the arthritic joints, mediated the antigen-induced flare-up.^{25,26} Antigen-stimulated T cells could either produce IL-1 themselves or stimulate IL-1 production in other cells. In rheumatoid arthritis, it is still a matter of debate whether the arthritic process was driven by T cells, immune complexes (IC), or both or by nonimmunological pathways. Our study clearly demonstrates that IL-1 plays a key role in the antigen-induced exacerbation of murine arthritis.

Materials and Methods

Animals

Female New Zealand White rabbits, male Wistar rats, and male C57Bl/6 mice (Jackson Laboratories, West Grove, PA) were fed a standard diet and tapwater *ad libitum*.

Cytokines

Murine recombinant IL-1 α and IL-1 β were a generous gift from I. G. Otterness (Pfizer Central Research, Groton CT).

Generation of Rabbit Anti-Mouse IL-1 Antiserum

Rabbits were immunized with murine recombinant IL-1 α or IL-1 β , according to the method described by Hogquist et al²⁷ with some modifications. In short, 250 μ g of IL-1 in 2.25 ml of phosphate-buffered saline (PBS) was emulsified with 500 μ l of Imject Alum (aluminum hydroxide; Pierce Chemical Co., Rockford, IL). Each rabbit received four subcutaneous injections (500 μ l each) of the IL-1/Alum suspension at the back of the animal and adjacent to each of these sites, four 500- μ l injections of complete Freund's adjuvant (C.F.A)/PBS subcutaneously. Each rabbit received three booster injection of 50 μ g of IL-1/Alum, adjacent injections with incomplete Freund's adjuvant emulsified in PBS, and additionally 5 μ g of IL-1 intravenously every 4 to 6 weeks. Ten days after every booster, 50 ml of blood were aspirated, coagulated, decomplemented at 56 C, and stored at -70 C. The neutralizing capacity was 300 μ g of IL-1 per ml of the pooled antiserum.

Purification and Characterization of Rabbit Anti-IL-1 Antibodies

Immunoglobulins were purified by affinity chromatographic separation on a protein G sepharose 4B (albumin binding region genetically deleted; Sigma Chemical Co., St Louis, MO) column. Immunoglobulins were eluted with 0.1 mol/L glycine-hydrochloride, pH 3.0, and immediately neutralized with 50 mmol/L Tris-HCl, pH 8.0. Fractions were pooled, concentrated, and dialyzed against PBS at room temperature (RT). The anti-IL-1 antibodies showed no neutralizing reactivity against the other IL-1 subtype, IL-2, IL-4, IL-6, or tumor necrosis factor.

Generation of Rat Anti-Mouse IL-1 Antiserum

Wistar rats were injected subcutaneously with 50 μ g of murine recombinant IL-1 β emulsified in 0.5 ml of CFA. After 2 weeks, a booster injection was given of 25 μ g of IL-1 in 0.5 ml of CFA. Rats were bled by orbital puncture at a regular basis 2 weeks after the booster injection.

Flare-Up of Arthritis

Mice of 8 to 12 weeks of age received subcutaneously 100 µg of mBSA (Sigma), emulsified in 100 µl of CFA, per animal. Injections were divided over both flanks and footpads of the forelegs. Heat-killed (2×10^9) *Bordetella pertussis* organisms (National Institute of Public Health, Bilthoven, The Netherlands) was administered intraperitoneally as an additional adjuvant. Mice received two booster injections of 50 µg of mBSA/CFA in the neck region on day 7. Three weeks later, arthritis was induced by injection of 6 µl containing 60 µg of mBSA in saline into the right knee joint cavity. At week 3 or 4 of arthritis, 0.2 to 2 µg of mBSA were injected intra-articularly into the arthritic joints to induce a flare-up of smoldering inflammation.

Joint Swelling

Mice were sedated by intraperitoneal injection of 0.25 ml of a 4.5% chloral hydrate solution per mouse. Approximately 10 µCi ^{99m}Tc pertechnetate (^{99m}Tc) in 0.2 ml of saline were injected subcutaneously in the neck region. After 15 minutes the accumulation of the isotope in the knee was determined by external gamma counting and expressed as the ratio of ^{99m}Tc uptake in the inflamed to ^{99m}Tc uptake in the contralateral knee joint. A ratio higher than 1.1 indicates joint swelling.

IL-1 Production by Synovial Tissue

Patellae were dissected with surrounding soft tissue consisting of the tendon and synovium in a standardized manner. Each patella was incubated in 200 µl of serum-free RPMI 1640 (Dutch modification) medium with Glutamax-1 (GIBCO BRL, Life Technologies, UK) for 1 hour at RT. Dilutions of these washouts were tested for IL-1 and IL-2 bioactivity.

Bioassay for IL-1

IL-1 activity was measured in the one-stage bioassay for IL-1 as described by Gearing et al²⁸ The murine thymoma cell line EL-4 NOB-1 (ECACC, Porton Down, Salisbury, UK) was used as an IL-1-specific cell producing IL-2 in response, in combination with the IL-2-sensitive CTLL-2 cells (ECACC). The cells were plated out in concentrations of 1×10^5 NOB1 cells per well and 4×10^3 CTLL cells per well in RPMI supplemented with 5% fetal calf serum. After 18 to 20 hour, 0.5 µCi of [^3H]thymidine (specific activity, 20 Ci/mmol; Dupont NEN Products, Boston, MA) were added per well. After 3 hours, cells were harvested and thymi-

dine incorporation (NOB1 cells are thymidine kinase deficient) was determined. Detection limit of the assay was 0.1 pg/ml murine recombinant IL-1.¹⁸

Assessment of Proteoglycan Synthesis

Patellae ($n = 6$), in a minimal amount of adjoining soft tissue (parts of synovium, tendon, and muscle), were placed in 2 ml of RPMI 1640 medium with gentamicin (50 µg/ml) and 40 µCi [^{35}S]sulfate. At the end of the 3-hour incubation period, patellae were fixed in 10% formalin and subsequently decalcified in formic acid (4%), dissected, and dissolved in 0.5 ml of Lumasolve (Hicol, Oud-Beijerland, The Netherlands). The ^{35}S content of each patella was measured by liquid scintillation counting and expressed as counts per minute (cpm) or as a percentage of normal cartilage.

Histology

Standard frontal sections (7 µ) of the whole knee joint, including both menisci, cruciate ligaments, and the patella, were prepared and mounted on 2% gelatin-coated slides as previously described. Slides were stained with safranin-O and counterstained with fast green. Cartilage depletion (diminished red staining) and inflammation were scored by a blinded observer.

Autoradiography

[^{35}S]sulfate (100 µCi) (specific activity, 1200 to 1400 Ci/mmol; Dupont NEN) was injected intraperitoneally 6 hours before dissection of the knee joints. After histological processing, 7-µ sections mounted on 0.5% gelatin-coated slides were dipped in K5 emulsion (Ilford Basildon, UK). The slides were developed and stained with hematoxylin and eosin after an exposure period of several weeks. PG synthesis was scored on the blackening of the chondrocytes by a blinded observer.

IL-1 Immunostaining

Paraffin-embedded sections were mounted on 2% gelatin-coated slides, deparaffinized in xylene for 5 minutes twice, and rehydrated to deionized water. Endogenous peroxidase activity was blocked with 1% H_2O_2 in PBS for 20 minutes at RT. Slides were then rinsed in water and incubated overnight with the primary antibody, rat anti-IL-1 β antiserum, or irrelevant rat antibody, diluted 1/150 in PBS plus 5% nonfat dry

milk at 4 C in a humidified box. Were washed in excess PBS plus 0.1% Tween 20, three times for 10 minutes each time and incubated with biotinylated secondary antibody, rabbit anti-rat antibody diluted 1/200 in PBS plus 5% nonfat dry milk and 2% normal mouse serum for 1 hour at RT. They were then washed again in PBS/Tween 20 three times for 10 minutes each time. Avidin-biotin-peroxidase conjugated complex was prepared according to the directions for preparation (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Slides were incubated with the ABC reagent diluted in PBS plus 5% nonfat dry milk for 30 minutes at RT and washed again in PBS/Tween 20. Slides were then developed with 0.5 mg/ml diaminobenzidine in Tris-HCl, pH 7.6, and 0.02% H₂O₂ for 10 minutes and counterstained with hematoxylin for 15 seconds. Anti-IL-1 antiserum was preincubated on IL-1 β -coated plates, eight times for 20 minutes each at RT, or normal rat serum was used as a negative control. For control of IL-1 β specificity, antiserum was preincubated on IL-1 α - or BSA-coated plates.

Treatment of Mice with Anti-IL-1 Antibodies

Mice received 200 μ l of a standard dose of 1 mg of purified rabbit anti-IL-1 α antibody and 1 mg of anti-IL-1 β antibody intravenously into the orbita plexus 1 hour before induction of the flare-up. These antibodies had an excellent half-life of more than 3 days in the blood circulation as was assessed by enzyme-linked immunosorbent assay. Total neutralizing capacity of the received dose was approximately 32 ng of both subtypes of IL-1 as was tested in the IL-1 bioassay (NOB-1) and blocked the effect of 1 ng of IL-1 α or - β on PG synthesis completely and that of 10 ng of IL-1 partially *in vivo* (not shown). Normal rabbit IgGs or polyclonal anti-ovalbumin antibodies served as controls for nonspecific effects of the treatment.

Results

IL-1 Expression in Antigen-Rechallenged Knee Joints of Chronic Arthritis

At the time of antigen rechallenge, week 3 of AIA, avidin-biotin immunohistochemistry with rat antiserum to IL-1 β failed to demonstrate IL-1 on whole knee joint sections (not shown). A single intra-articular injection of a small amount of antigen (2 μ g of mBSA) caused a transient expression of cell-associated IL-1 β . The number of IL-1 β -positive cells was small in the joint sections taken at 1 hour, high at

3 hours, low but still evident at 6 and 12 hours, and absent at 24 hours after the antigen rechallenge (not shown). The IL-1 β signal was predominantly localized in the synovial lining layer and frequently in focal areas in the inflamed synovium (Figure 1). No immunostaining was evident with normal rat serum or with anti-IL-1 β antiserum preincubated on microtiter plates coated with murine recombinant IL-1 β (Figure 1). In some sections, chondrocytes and the exudate PMNs were nonspecifically stained.

A single intra-articular injection of mBSA into hypersensitive joints (week 3 of AIA) caused a dose-related joint swelling at 6 hours (Figure 2). Synovial tissue culture supernatants demonstrated increased levels of IL-1 bioactivity 6 hours after antigen rechallenge in the tested dose range of 0.06 to 2 μ g of mBSA (Figure 2). Time course experiments demonstrated a rapid onset of joint swelling increasing at least up to 24 hours after antigen (2 μ g mBSA) rechallenge of AIA (Figure 3). The level of bioactive IL-1 in synovial tissue culture supernatants of arthritic joints was high 3 and 6 hours after antigen rechallenge (Figure 3). The expression of IL-1 was more transient compared with joint swelling. At 24 hours after antigen injection, IL-1 released by the synovial tissue was low yet joints were profoundly swollen. Joint swelling was not inducible with 2 μ g of mBSA injected into naive joints of sensitized animals (Figure 3). Interestingly, the antigen-challenged naive joints released IL-1 in levels and time course comparable with the rechallenged arthritic joints (Figure 3).

Role of IL-1 in the Antigen-Induced Exacerbation of Chronic Joint Inflammation

At the time of antigen rechallenge, week 3 of AIA, swelling of the arthritic joints subsided and the inflammatory exudate cells disappeared, but joints had a mild ongoing synovitis (Table 1). Intra-articular injection of 2 μ g of mBSA into the arthritic joints caused an acute transient joint swelling (Table 1). On day 2 of the flare-up a Tc ratio of 1.22 ± 0.14 was measured (not shown). The same antigen dose injected into the naive contralateral joints of these arthritic animals did not cause joint swelling (Table 1).

To investigate the role of *de novo* synthesized IL-1, neutralizing polyclonal anti-IL-1 α and - β antibodies were injected intravenously 1 hour before antigen rechallenge. Blocking IL-1 decreased joint swelling by 29% at 6 hours and significantly reduced joint swelling by 41% at 24 hours after antigen rechallenge as compared with untreated mice (Table 1). This was a

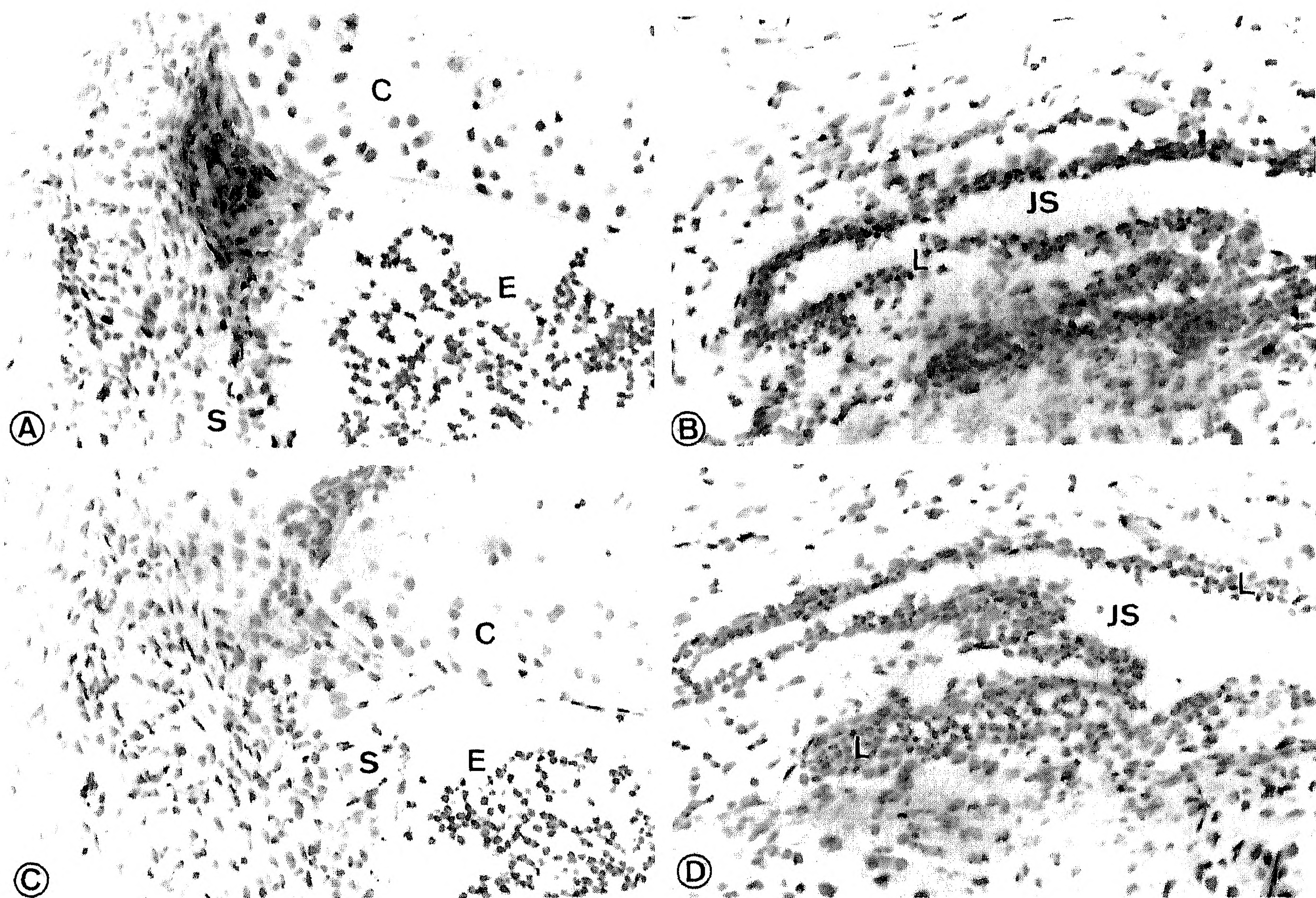


Figure 1. Immunoperoxidase localization of whole knee joint sections showing cells stained for IL-1 β (magnification, $\times 250$) in focal areas of the inflamed synovium (A) and in the synovial lining layer (B). Antigen (2 μ g of mBSA) was injected intra-articularly at week 3 of AIA and joints were dissected 3 hours thereafter. Serial sections stained with normal rat serum (C) or with anti-IL-1 β antiserum preincubated on IL-1 β -coated microtiter plates (D) served as negative controls. C, cartilage; E, exudate; JS, joint space; L, lining layer; S, synovium.

consistent finding in all experiments: mean joint swelling of 1.60 ± 0.21 in the untreated mice ($n = 47$), 1.52 ± 0.24 in the normal rabbit IgG-treated mice ($n = 68$), and 1.37 ± 0.29 in the anti-IL-1-treated mice ($n = 75$) on day 1 after antigen rechallenge.

Antigen injection also caused a marked influx of polymorphonuclear neutrophils (PMN) into the cavity and exacerbated the ongoing synovitis in the hypersensitive joint (Table 1). A moderate inflammation was inducible in the naive contralateral joints of these sensitized animals with the same antigen dose (2 μ g of mBSA) used (Table 1).

Anti-IL-1 α and - β antibody pretreatment had no effect on the number of inflammatory cells (PMN) in the 6- and 24-hour exudate and on the extent of the synovitis at these time points (Table 1). A marked amelioration of the synovitis was observed on day 4 after antigen rechallenge in the anti-IL-1-treated group (Table 1 and Figure 4). This was a consistent finding in three out of a total of four experiments. Synovitis in the normal rabbit IgG-treated mice (1.55 ± 0.69 , $n = 21$) was significantly higher than in the anti-IL-1-treated mice (0.63 ± 0.23 , a reduction of 59%).

Role of IL-1 in the Flare-Up Related Cartilage Pathology

Chondrocyte PG synthesis, markedly suppressed for the first weeks of AIA, returned to a normal rate at week 3 of AIA. A marked suppression of PG synthesis was evident on day 1 (-28%) and day 2 (-23%) after antigen rechallenge in patellar cartilage as was measured by [35 S]sulfate incorporation *ex vivo* (Table 2). Pretreatment of mice with anti-IL-1 antibodies completely prevented the PG synthesis suppression to occur (Table 2). Autoradiography of whole knee joint sections also demonstrated reduced [35 S]sulfate incorporation by chondrocytes in the cartilage of arthritic joints from day 1 up to day 4 after antigen rechallenge (Table 3 and Figure 5). Blocking IL-1 activity with antibodies significantly enhanced PG synthesis in antigen-rechallenged mice (Table 3 and Figure 5). Interestingly, injection of 2 μ g of mBSA into the naive contralateral joints of these mice also resulted in a significant suppression of PG synthesis on day 1 (Tables 2 and 3).

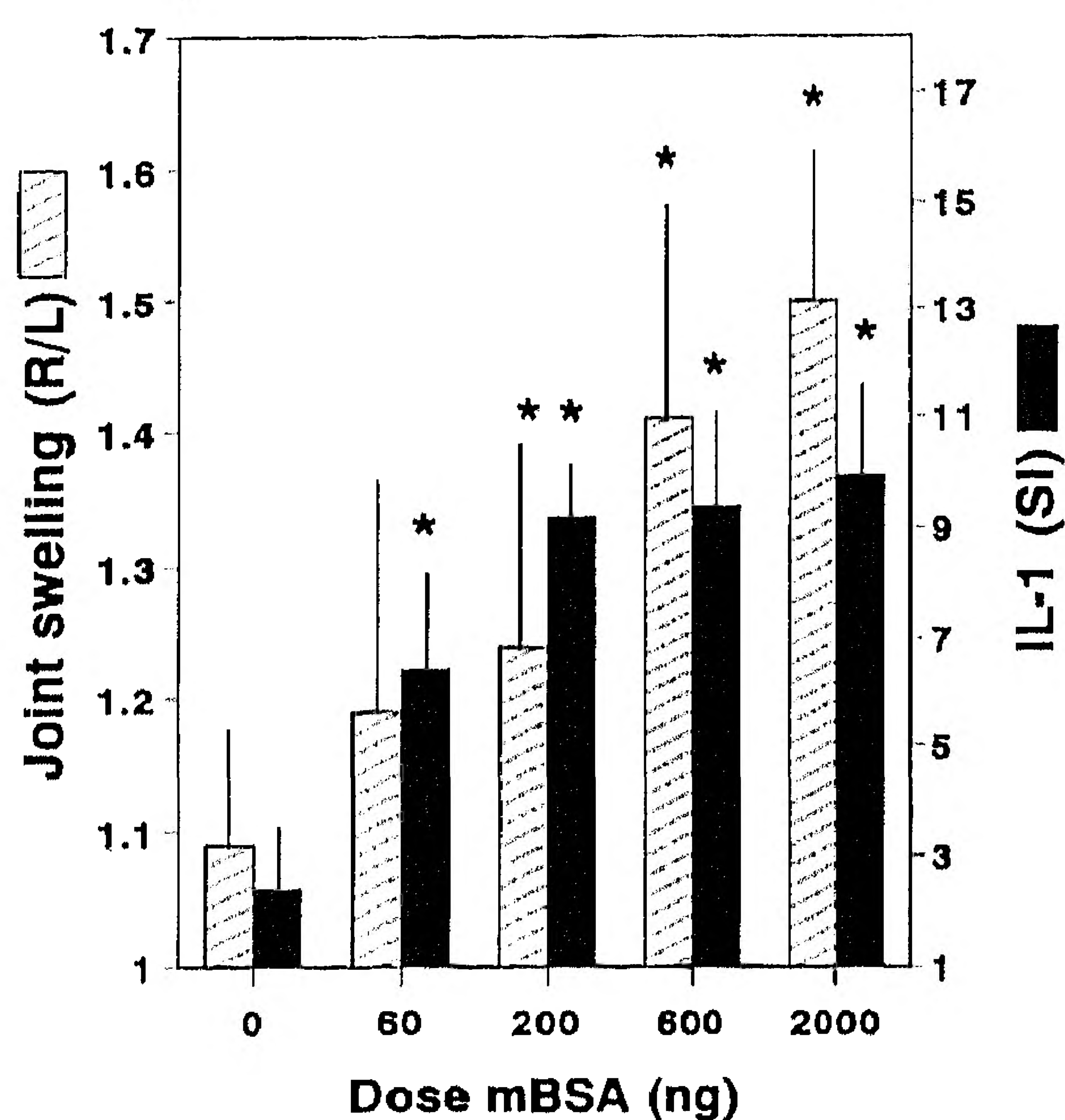


Figure 2. Dose-related joint swelling and IL-1 production in antigen-rechallenged hypersensitive knee joints. Antigen (0.06 to 2 μ g of mBSA) was injected intra-articularly into arthritic joints at week 3 of AIA. Joint swelling was measured 6 hours after the antigen challenge by external gamma counting of 99m Tc uptake. Values represent the mean ratio of arthritic (R) over the naive contralateral joint (L) \pm SD of seven animals. A 1/80 dilution of culture supernatants of synovial tissue taken 6 hours after antigen rechallenge was tested for IL-1 by a cell proliferation assay. Values represent the mean stimulation index \pm SD of six individual synovia. Highest signal (SI) is comparable with the response to 4 μ g/ml IL-1 in the assay (10,000 cpm). Statistical significance was tested by using the Wilcoxon's rank sum tests. * $P < 0.05$ was considered significant.

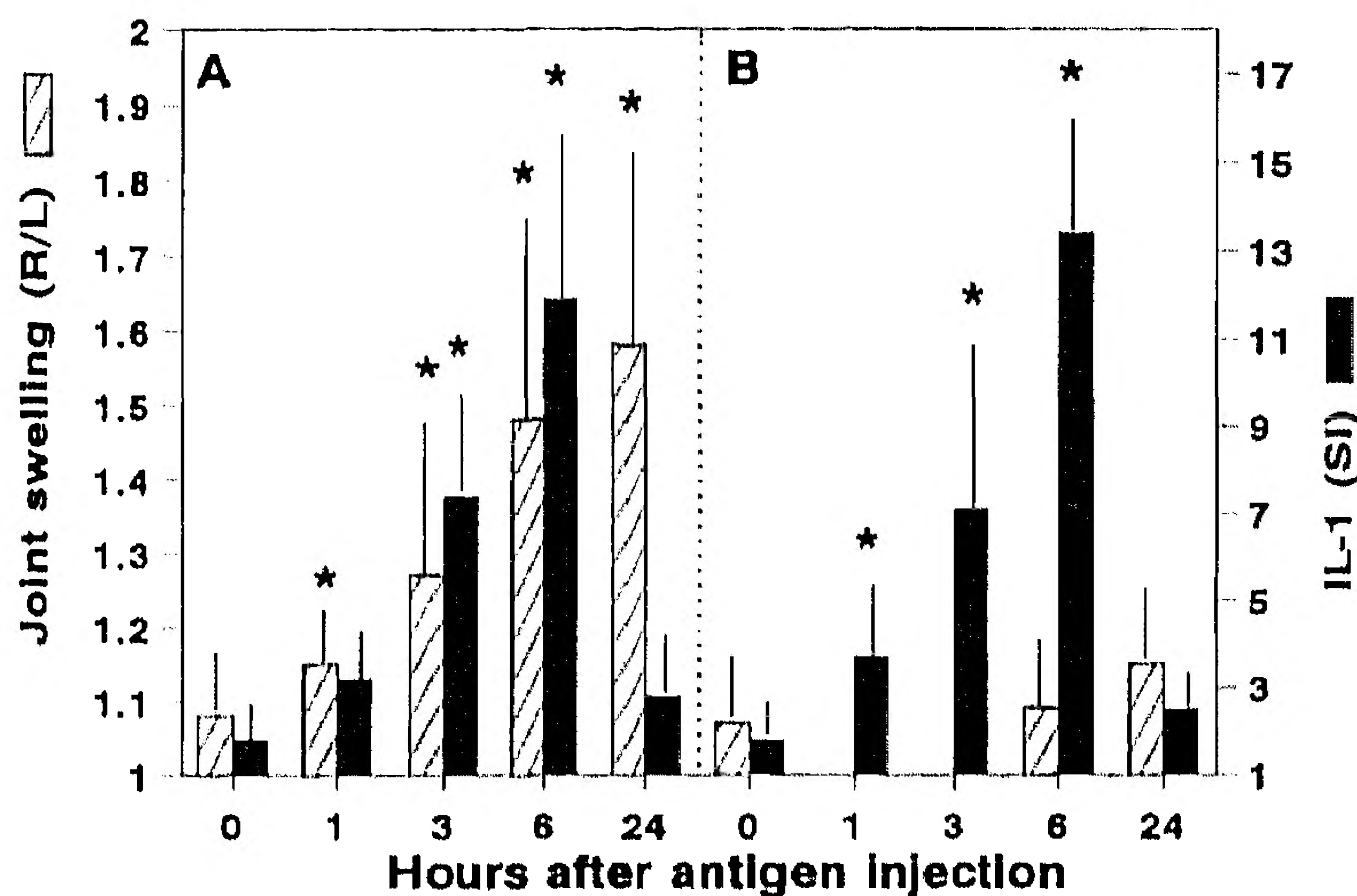


Figure 3. Time-related changes in joint swelling and IL-1 production in antigen-rechallenged hypersensitive knee joints (A) and in naive joints of sensitized mice (B). Antigen (2 μ g of mBSA) was injected intra-articularly and both joint swelling and IL-1 production by the synovial tissues was measured (as described in Figure 2) at several time points thereafter in six animals each. Statistical significance was tested by using the Wilcoxon's rank sum tests. * $P < 0.05$ was considered significant.

Enhanced PG degradation (-59%) was found in the hypersensitive joints 1 day after antigen rechallenge as was measured on [35 S]sulfate prelabeled patellar cartilage *in vivo* (Table 2). The accelerated degradation could be significantly reduced by anti-IL-1 treatment but was still remarkable (-37% Table 2).

Histology taken at week 4, but not at week 3, of AIA showed full recovery of the cartilage matrices (Figure

4). Antigen (2 μ g of mBSA) rechallenge caused severe PG depletion of the cartilage matrices as was shown on safranin-O-stained whole knee joint sections (Table 3 and Figure 4). Pretreatment with anti-IL-1 antibodies, but not with control IgG, significantly ameliorated the cartilage pathology on day 4 of the antigen-induced exacerbation (Table 3 and Figure 4). Anti-IL-1 α and - β pretreatment completely prevented the antigen-induced cartilage depletion in the hypersensitive joints in four of five experiments. Antigen injection into the naive contralateral joints of the arthritic mice caused only minor cartilage depletion (Table 3).

Discussion

This study clearly demonstrates that IL-1 is an important determinant in antigen-induced exacerbations of murine arthritis. In the conducted experiments, pretreatment of mice with rabbit polyclonal antibodies directed against murine recombinant IL-1 α and IL-1 β markedly alleviated joint swelling and ameliorated cartilage pathology of the antigen-rechallenged joints.

Using avidin-biotin immunohistochemistry, we showed IL-1 β expression in the synovium of antigen-rechallenged joints, predominantly located in the lining layer and in distinct cell clusters in the sublining layer and in single cells sparsely distributed in the deeper area. An identical localization was also found in synovial membranes of rheumatoid arthritis for IL-1 α and IL-1 β .^{1,2} The expression of IL-1 α was identical to IL-1 in the rheumatoid synovium although the number of positive cells was considerably smaller.¹ We found identical kinetics for the expression of both bioactive and immunoreactive IL-1 in hypersensitive joints after antigen rechallenge. This indicates that IL-1 inhibitors (eg, IL-1ra), although they may be produced, were not responsible for the transient expression of bioactive IL-1 in the flare-up.

IL-1 was inducible in both virgin and hypersensitive joints of sensitized mice but not in joints of normal mice.¹⁸ This shows that the stimulation of IL-1 was not a property of the antigen but that either antigen-specific T lymphocytes or immunoglobulins were involved. IL-1 production in rheumatoid synovial membranes was considerably higher in membranes possessing lymphocyte aggregates compared with membranes with a disperse infiltrate.²⁹ These focal areas are composed of B cells, plasma cells, and CD4 $^{+}$ T cells in the center and macrophages at the periphery. Not only T helper type 2 cells but also B cells, as almost all accessory cells (eg, macrophages), can produce IL-1. We did not quantitatively

Table 1. Role of IL-1 in the Antigen-Induced Exacerbation of Smoldering Joint Inflammation

Antigen*	Pretreatment	Joint swelling To ratio†		Histological analysis				
		6 hours (n = 8)	24 hours (n = 12)	Exudate‡		Infiltrate		Synovial thickness
				6 hours (n = 7)	24 hours (n = 8)	6 hours (n = 7)	24 hours (n = 7)	96 hours (n = 8)
None	None	1.07 ± 0.07	1.05 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.6	0.4 ± 0.4	0.4 ± 0.2
+	None	1.49 ± 0.26	1.70 ± 0.10	1.4 ± 0.5	1.7 ± 0.9	1.7 ± 0.8	1.6 ± 0.8	1.4 ± 0.9
+	Normal rabbit serum	1.46 ± 0.15	1.66 ± 0.20	1.8 ± 0.9	1.8 ± 0.8	2.1 ± 1.1	1.9 ± 0.6	1.4 ± 0.9
+	Anti-IL-1α and -β	1.35 ± 0.23	1.41 ± 0.17§	1.6 ± 1.0	1.8 ± 0.8	2.1 ± 1.2	1.6 ± 0.8	0.5 ± 0.1§
+	None	ND	1.06 ± 0.08	0.3 ± 0.3	0.6 ± 0.4	0.2 ± 0.3	0.4 ± 0.1	ND

* Arthritic knee joints rechallenged with 2 µg of mBSA at week 3 of AIA (+). For the last row, antigen was injected into the naive contralateral knee joints.

† Joint swelling was measured at 6 and 24 hours after antigen injection by To uptake. Results are expressed as ratio of uptake in inflamed to uptake in contralateral knee joint ± SD.

‡ Histological analysis of cell exudate (PMNs) in the joint cavity at 6 and 24 hours after antigen injection. Arbitrary scoring from 0 (no cells) to 3 (large number of inflammatory cells) by a blinded observer.

§ $P < 0.05$ was considered significant, as tested by using the Wilcoxon's rank sum tests.

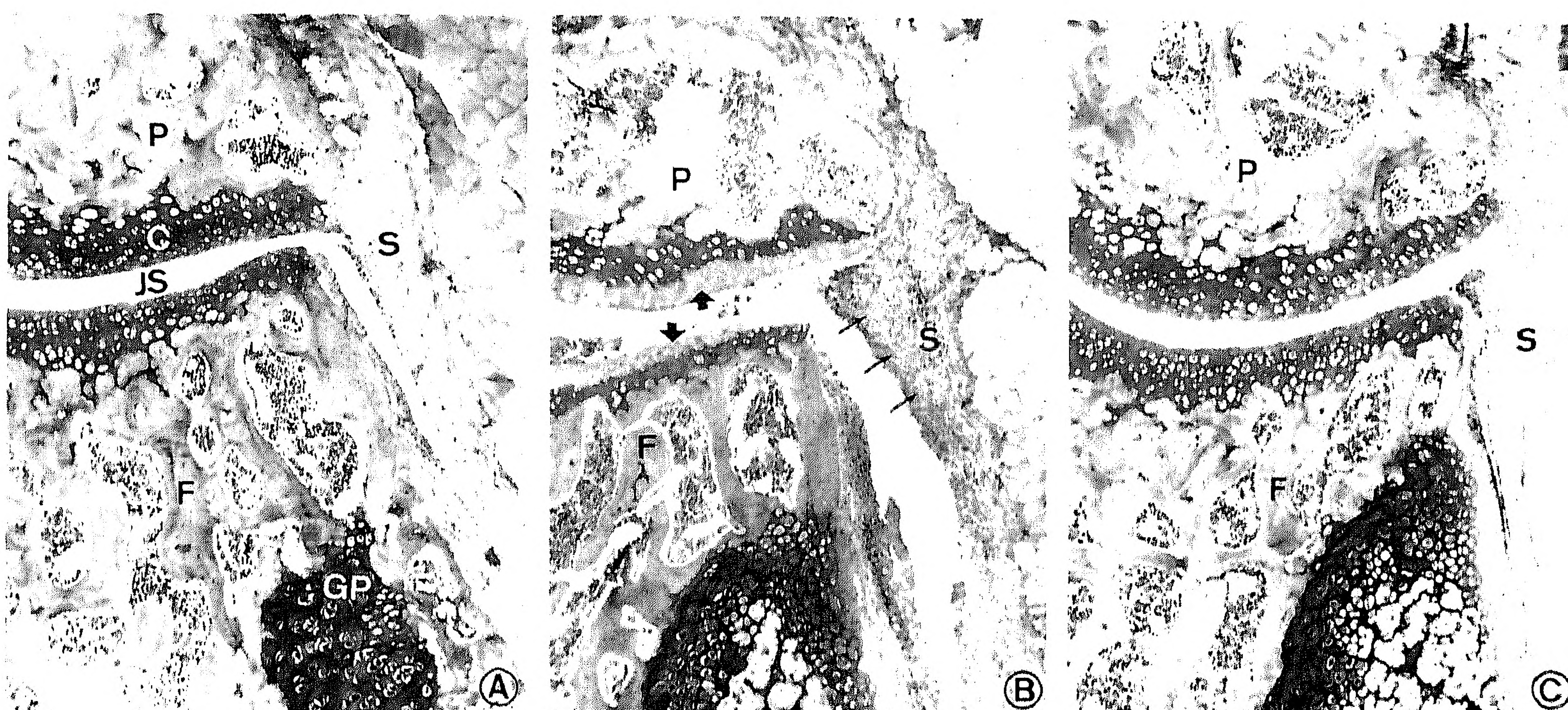


Figure 4. Histology of whole knee joint sections stained with safranin-O. A Arthritic knee joint at week 4 of AIA. B Arthritic joint in a normal rabbit IgG-treated mouse 4 days after antigen challenge (2 µg of mBSA). Note the thickening of the synovium (thin arrow) and PG loss in the cartilage matrix (compact arrow). C Antigen-rechallenged joint in an anti-IL-1 antibody-treated mouse. Original magnification, ×100. P, patella; F, femur; JS, joint space; S, synovium; C, cartilage.

compare the IL-1 production in antigen challenged naive and hypersensitive joints, but only in the latter did we find distinct staining of cell clusters.

The antigen dose used for the flare-up of AIA did not induce inflammation when injected in normal knee joints of sensitized mice. This suggests that the antigen-induced IL-1 production was too low to induce joint inflammation in normal joints. This is in agreement with a previous study of ours in which we showed that a single injection of 10 ng of IL-1 did not induce joint swelling or cell exudate in naive joints but induced a brief flare-up of the inflammation in arthritic joints of AIA.^{8,30} A highly likely explanation for this hypersensitive state could be the increased expres-

sion of adhesion molecules (VCAM-1 and intercellular adhesion molecules) in the inflamed synovium.³¹

In the present study we found, using anti-IL-1α and -β antibodies, that the cell exudate and the joint swelling for the greater part (60 to 70%) were not IL-1 mediated in the antigen-induced exacerbation of AIA, and this suggests that other mediators are involved in this process. Possible candidates are T cell-derived mediators. Evidence was presented that CD4⁺ T cells are mediating the antigen-induced exacerbation of murine arthritis.³² It was concluded from the full blockade of the flare-up by anti-Ia (histocompatibility type II antigens) antibodies that antigen presentation was of importance.²⁶ Although IL-1 is capable of ac-

Table 2. *Effect of Anti-IL-1 Treatment on PG Synthesis Suppression and PG Degradation in Antigen-Challenged Joints*

Antigen*	Treatment†	PG metabolism (cpm ± SD)		
		Synthesis day 1 (n = 7)‡	Synthesis day 2 (n = 7)‡	Degradation day 1 (n = 6)§
None	None	1118 ± 104	1238 ± 289	360 ± 99
+	None	739 ± 54 (-28%)	958 ± 251 (-23%)	ND
+	NRS	732 ± 103 (-35%)	913 ± 343 (-26%)	148 ± 39 (-59%)
+	Anti-IL-1	1068 ± 143 (-4%)	1519 ± 778 (+23%)	226 ± 40 (-37%)
None	None	1153 ± 258	ND	ND
+	None	860 ± 170 (-25%)	ND	ND

* Arthritic knee joints rechallenged with 2 µg of mBSA at week 3 of AIA (+). For the last two rows, antigen was injected into the naive contralateral knee joints.

† Normal rabbit antibodies (NRS) or anti-IL-1α and -β antibodies injected 1 hour before antigen rechallenge.

‡ [³⁵S]Sulfate incorporation into patellar cartilage *ex vivo*. Values in parentheses indicate the inhibition of PG synthesis as a percentage of the unchallenged joint. Representative of two experiments.

§ [³⁵S]Sulfate content in patellar cartilage. [³⁵S]Sulfate was injected subcutaneously 24 hours before antigen injection.

|| *P* < 0.05 was considered significant, as tested by using the Student *t*-test.

Table 3. *Histological Analysis of the Anti-IL-1 Treatment Effect on PG Synthesis and Cartilage PG Depletion in Antigen-Challenged Joints*

Antigen*	Treatment	PG synthesis†			PG depletion‡
		Day 1 (n = 7)	Day 2 (n = 8)	Day 4 (n = 6)	Day 4 (n = 8)
None	None	2.0 ± 0.9	2.2 ± 0.3	2.1 ± 0.9	0.4 ± 0.8
+	None	0.5 ± 0.3 (-77%)	ND	ND	2.1 ± 0.6
+	NRS	0.7 ± 0.4 (-70%)	0.9 ± 0.6 (-60%)	0.9 ± 0.7 (-60%)	1.9 ± 0.9
+	Anti-IL-1	1.4 ± 0.7 (-30%)	1.9 ± 0.7 (-14%)	2.5 ± 0.5 (+17%)	0.3 ± 0.5
None	None	1.3 ± 0.6	ND	ND	0.0 ± 0.0
+	None	0.5 ± 0.3 (-63%)	ND	ND	0.4 ± 0.2

* Arthritic knee joints rechallenged with 2 µg of mBSA at week 4 of AIA (+). For the last two rows antigen was injected into the naive contralateral knee joints.

† Normal rabbit antibodies (NRS) or anti-IL-1α and -β antibodies injected 1 hour before antigen rechallenge.

‡ Autoradiography of whole knee joint sections. [³⁵S]Sulfate incorporation into patellar cartilage *in vivo* as scored arbitrarily from 0 (no black spots) to 3 (strong blackening as seen in the growth plates). Values in parentheses indicate PG synthesis inhibition as a percentage of the unchallenged joint.

§ Histological analysis of patellar cartilage PG depletion as scored arbitrarily from 0 (strong safranin-O staining, no depletion) to 3 (unstained cartilage, strong depletion) by a blinded observer.

|| *P* < 0.05 was considered significant, as tested by using the Wilcoxon's rank sum tests.

tivating lymphocytes, we found that the IL-1-induced exacerbation of AIA was not lymphocyte mediated, and this implies that the antigen- and IL-1-induced flare-up are different from each other.³⁰ Evidence is emerging that IL-1 does not play a prominent role in antigen-induced T cell activation.^{33,34} We selectively blocked the Th1-derived IL-2 and the Th2-derived IL-4 with antibodies and this markedly reduced joint swelling, cell exudate, and infiltrate during the antigen-induced flare-up.³² Co-injection of large amounts of IL-2 or IL-4 with the antigen significantly enhanced cell exudate. We concluded that the T cell-derived cytokines IL-2 and IL-4 are acting as proinflammatory cytokines during antigen-induced exacerbation of AIA.

Schwab et al³⁵ demonstrated that reactivation of streptococcal cell wall-induced arthritis by the T cell superantigen, toxic shock syndrome toxin 1, was neither IL-1 nor tumor necrosis factor-α dependent, in contrast with the peptidoglycan polysaccharide reactivation, suggesting that the latter was not solely T

cell mediated. A more important role of IL-1 was found in IC-mediated inflammations.^{36,37} Recently we developed an IC-mediated arthritis model (ICA) by passive immunization in the mouse.¹⁴ In this model, IL-1 and complement activation acted synergistically in the onset of inflammation. Striking anti-inflammatory effects with anti-IL-1 antibodies or IL-1 receptor antagonist protein (IL-1ra) were also found in CIA, even when treatment was started in already established disease.¹³ In contrast, anti-IL-1 treatment had no effect on the onset of AIA.¹⁸⁻²⁰ CIA is probably more IC than T cell mediated.^{38,39} For instance, although CIA could be transferred by T cells or IC, a combination resulted in a more pronounced inflammation.⁴⁰ However, AIA and the subsequent hypersensitive state of the joints could be transferred with antigen (mBSA)-specific T cells to naive recipients.⁴¹ Up until now, we were unable to transfer the disease (AIA) by B cells or immunoglobulins (unpublished data). The importance of IL-1 could be related to the type or the site of the inflammation.^{16,37}

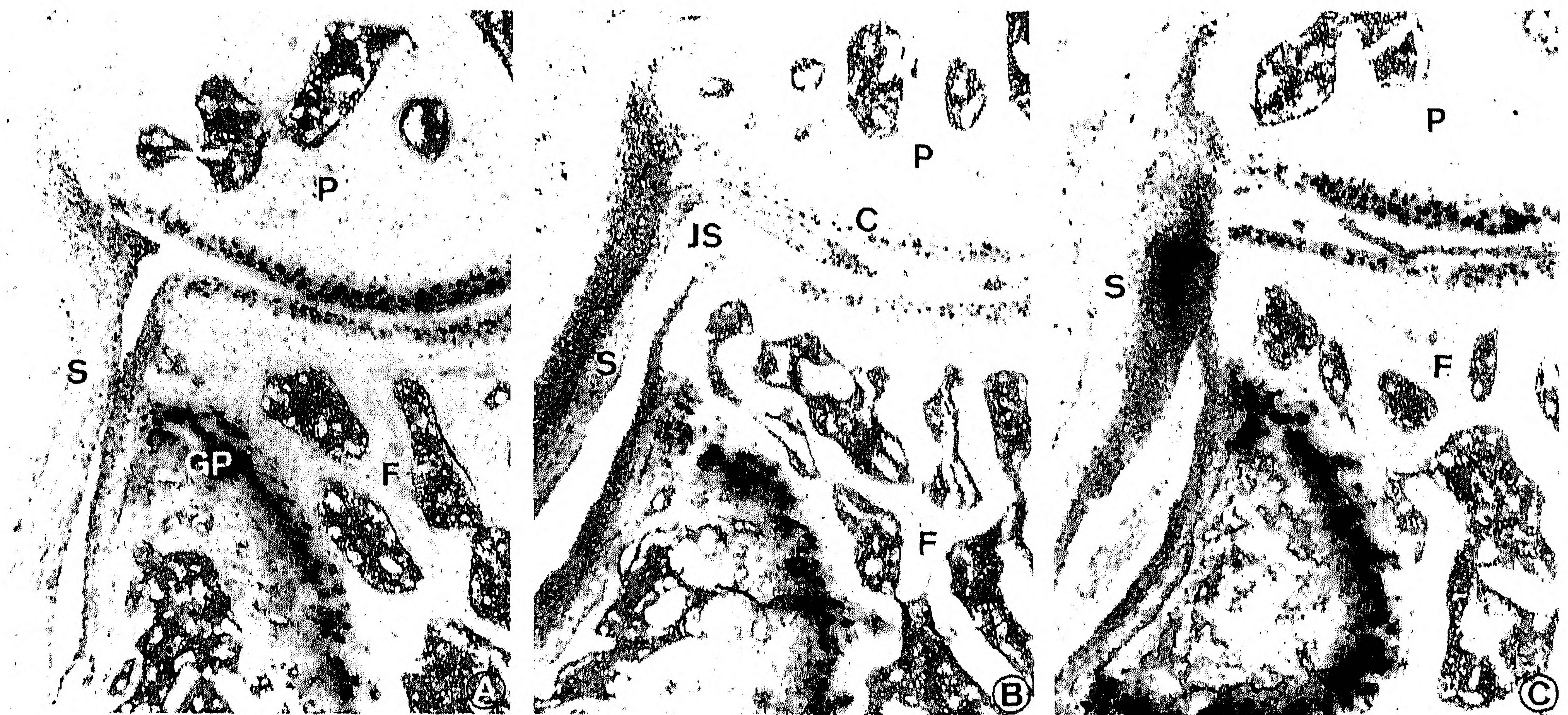


Figure 5. Autoradiographic analysis of chondrocyte PG synthesis. Mice received 75 μ Ci $[^{35}\text{S}]$ sulfate subcutaneously and 4 hours later the knee joints were dissected and processed for histology. **A** Arthritic joint at week 3 of AIA, background control. **B** Arthritic joint of normal rabbit IgG-treated mouse 1 day after antigen rechallenge (2 μ g of mBSA). **C** Antigen-rechallenged arthritic joint in an anti-IL-1 antibody-treated mouse. $[^{35}\text{S}]$ Sulfate incorporation is indicated by the black spots in cartilage and growth plates. Original magnification, $\times 100$. P, patella; F, femur; JS, joint space; S, synovium; C, cartilage; GP, growth plate.

Antigen rechallenge of the hypersensitive joints induced a marked inhibition of chondrocyte PG synthesis for at least 4 days. This was not a property of the antigen as PG synthesis suppression was not inducible in joints of nonimmune mice.¹⁸ The flare-up of the inflammation was also not responsible for the suppression of the chondrocyte synthetic function for two reasons. First, antigen injection into naive joints of sensitized mice induced both IL-1 production and inhibition of PG synthesis without a significant joint inflammation. Second, blocking IL-1 with neutralizing anti-IL-1 α and - β antibodies protected the chondrocyte synthetic function without affecting the onset of the flare-up. We previously demonstrated a key role of IL-1 in the inhibition of PG synthesis in several murine arthritis models, ie, CIA, AIA, and ICA.^{13,18,19,42}

The enhanced cartilage degradation in the antigen-challenged arthritic joints was, for approximately 50%, IL-1 mediated. In ICA and CIA, the anti-IL-1 also significantly reduced PG degradation, but this was probably related to the alleviation of joint inflammation.^{13,43} A more likely explanation for the protective effect of anti-IL-1 treatment on the flare-up is a higher vulnerability for IL-1-induced degradation of cartilage in the recovery phase of arthritis (manuscript in preparation). Pelletier et al⁴⁴ presented evidence for an enhanced number of IL-1 receptors on chondrocytes in human osteoarthritic cartilage and showed that the IL-1 required for half-maximal metalloproteinase stimulation was three to four times lower compared with normal cartilage.

Protection of the chondrocyte synthetic function and reduction of the enhanced PG degradation probably resulted in the observed amelioration of the cartilage pathology on day 4 after antigen rechallenge. Mini-osmotic pumps releasing 30 μ g of IL-1ra per hour into the peritoneal cavity from day 2 before antigen rechallenge up to 7 days thereafter verified the anti-IL-1 antibody effects (not shown).

Another impressive effect of the anti-IL-1 treatment was the reduction in the synovial thickness (hyperplasia) on day 4 of the exacerbation. IL-1 is a potent growth factor for synovial fibroblasts, which can explain this anti-IL-1 effect.⁴⁵ Synoviocytes can actively participate in the local hypersensitivity for a number of reasons. First, synovial fibroblasts (synoviocytes) are accessory cells by their capacity of antigen presentation and IL-1 expression.^{46,47} Second, adhesion molecules can be induced by cytokines on type B synoviocytes, which may facilitate leukocyte recruitment.^{48,49} Moreover, unrestricted synoviocyte proliferation caused arthritis in animals and synovial tissue implants caused cartilage destruction.^{50,51} Therefore, the reduction of the synovial hyperplasia in the antigen-rechallenged joints could be responsible for the amelioration of arthritis.

This study indicates that IL-1 is involved as key mediator in the pathological changes of cartilage, not only in IC-mediated inflammation but also in T cell-mediated inflammation. Our study strengthens the need for additional research of the role of IL-1 even when IL-1 is not involved in the onset of inflammation.

References

- Deleuran BW, Chu CQ, Field M, Brennan FM, Katsiki P, Feldmann M, Maini RN: Localization of interleukin-1 α , type 1 interleukin-1 receptor and interleukin-1 receptor antagonist in the synovial membrane and cartilage/pannus junction in rheumatoid arthritis. *Br J Rheumatol* 1992, 31:801-809
- Farahat MN, Yanni G, Poston R, Panayi GS: Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis* 1993, 52:870-875
- Eastgate JA, Symons JA, Wood NC, Grinlinton FM, Di Giovine FS, Duff GW: Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. *Lancet* 1988, 24:706-709
- Feldmann M, Brennan FM, Chantry D, Haworth C, Turner M, Abney E, Buchan G, Barrett K, Barkley D, Chu A, Field M, Maini RN: Cytokine production in the rheumatoid joint: implications for treatment. *Ann Rheum Dis* 1990, 49:480-486
- Kahle P, Saal JG, Schaudt K, Zacher J, Fritz P, Pawelec G: Determination of cytokines in synovial fluids: correlation with diagnosis and histomorphological characteristics of synovial tissue. *Ann Rheum Dis* 1992, 51:731-734
- Pettipher ER, Higgs GA, Henderson B: Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. *Proc Natl Acad Sci USA* 1986, 83:8749-8753
- Chandrasekhar S, Harvey AK, Hurbey PS, Bendele AM: Arthritis induced by interleukin-1 is dependent on the site and frequency of intraarticular injection. *Clin Immunol Immunopathol* 1990, 55:382-400
- Van de Loo AAJ, van den Berg WB: Effects of murine recombinant interleukin 1 on synovial joints in mice: measurements of patellar cartilage metabolism and joint inflammation. *Ann Rheum Dis* 1990, 49:238-245
- Arner E, Pratta MA: Independent effects of interleukin-1 on proteoglycan breakdown, proteoglycan synthesis, and prostaglandin E₂ release from cartilage in organ culture. *Arthritis Rheum* 1989, 32:288-297
- Saklatvala J: Tumor necrosis factor α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature* 1986, 322:547-549
- Van den Berg WB, van de Loo FAJ, Zwarts WA, Otterness IG: Effects of murine recombinant interleukin-1 on intact homologous articular cartilage: a quantitative and autoradiographic study. *Ann Rheum Dis* 1988, 47:855-863
- Wooley PH, Whalen JD, Chapman DL, Berger AE, Richard KA, Aspar DG, Staite ND: The effect of an interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigen-induced arthritis in mice. *Arthritis Rheum* 1993, 36:1305-1314
- Van den Berg WB, Joosten LAB, Helsen M, van de Loo FAJ: Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment. *Clin Exp Immunol* 1994, 95:237-243
- Van Lent PLEM, van den Bersselaar LAM, van den Hoek AEM, van de Loo AAJ, van den Berg WB: Cationic immune complex arthritis in mice - a new model: synergistic effect of complement and interleukin-1. *Am J Pathol* 1992, 140:1451-1461
- Barak V, Peritt D, Flechner I, Sherman Y, Okon E, Yania P, Halperin T, Treves AJ: The M20 IL-1 inhibitor prevents onset of adjuvant arthritis. *Biotherapy* 1992, 4:317-323
- Issekutz AC, Meager A, Otterness I, Issekutz TB: The role of tumour necrosis factor- α and IL-1 in polymorphonuclear leucocyte and T lymphocyte recruitment to joint inflammation in adjuvant arthritis. *Clin Exp Immunol* 1994, 97:26-32
- Schorlemmer HU, Kanzy EJ, Langer KD, Kurrle R: Immunomodulatory activity of recombinant IL-1 receptor (IL-1-R) on models of experimental rheumatoid arthritis. *Agents Actions* 1993, 39:C113-C116
- Van de Loo FAJ, Arntz OJ, Otterness IG, van den Berg WB: Protection against cartilage proteoglycan synthesis inhibition by anti-interleukin 1 antibodies in experimental arthritis. *J Rheumatol* 1992, 19:348-356
- Van de Loo FAJ, Arntz OJ, Otterness IG, van den Berg WB: Modulation of cartilage destruction in murine arthritis with anti-IL-1 antibodies. *Agents Actions* 1993, 39:C211-C215
- Lewthwaite J, Blake SM, Hardingham TE, Warden PJ, Henderson B: The effect of recombinant human interleukin 1 receptor antagonist on the induction phase of antigen induced arthritis in the rabbit. *J Rheumatol* 1994, 21:467-472
- Bennet JC: The infectious etiology of rheumatoid arthritis. *Arthritis Rheum* 1978, 21:531
- Shore A, Jaglal S, Keystone EC: Enhanced interleukin 1 generation by monocytes *in vitro* is temporally linked to the early events in the onset or exacerbation of rheumatoid arthritis. *Clin Exp Immunol* 1986, 65:293-302
- Schwab JH, Anderle SK, Brown RR, Dalldorf FG, Thompson RC: Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. *Infect Immun* 1991, 59:4436-4442
- Stimpson SA, Esser RE, Carter PB, Sartor RB, Cromartie WJ, Schwab JH: Lipopolysaccharide induces recurrences of arthritis in rat joints previously injured by peptidoglycan-polysaccharide. *J Exp Med* 1987, 165:1688-1702
- Lens JW, van den Berg WB, van de Putte LBA, Berden JHM, Lems SPM: Flare-up of antigen-induced arthritis in mice after challenge with intravenous antigen: effects of pre-treatment with cobra venom factor and anti-lymphocyte serum. *Clin Exp Immunol* 1984, 57:520-528
- Van den Broek MF, van den Berg WB, van de Putte LBA: Monoclonal anti-Ia antibodies suppress the flare up reaction of antigen induced arthritis in mice. *Clin Exp Immunol* 1986, 66:320-330
- Hogquist KA, Nett MA, Sheehan KCF, Pendleton KD, Schreiber RD, Chaplin DD: Generation of monoclonal

- antibodies to murine IL-1 β and demonstration of IL-1 *in vivo*. *J Immunol* 1991, 146:1534-1540
28. Gearing AJH, Bird CR, Bristow A, Poole S, Thorpe R: A simple sensitive bioassay for interleukin-1 which is unresponsive to 10³ U/ml of interleukin-2. *J Immunol Methods* 1987, 99:7-11
29. Yanni G, Whelan A, Feighery C, Quinlan W, Symons J, Duff J, Breshnihan B: Contrasting levels of *in vitro* cytokine production by rheumatoid synovial tissues demonstrating different patterns of mononuclear cell infiltration. *Clin Exp Immunol* 1993, 93:387-395
30. Van de Loo AAJ, Arntz OJ, van den Berg WB: Flare-up of experimental arthritis in mice with murine recombinant IL-1. *Clin Exp Immunol* 1992, 87:196-202
31. Postigo AA, García-Vicuña R, Laffón A, Sánchez-Madrid F: The role of adhesion molecules in the pathogenesis of rheumatoid arthritis. *Autoimmunity* 1993, 16:69-76
32. Jacobs MJM, van den Hoek AEM, van Lent PLEM, van de Loo FAJ, van de Putte LBA, van den Berg WB: Role of IL-2 and IL-4 in exacerbations of murine arthritis. *Immunology* 1994, 83:390-396
33. Nicod LP, el-Habre F, Dayer JM: Natural and recombinant interleukin 1 receptor antagonist does not inhibit human T-cell proliferation induced by mitogens, soluble antigens or allogeneic determinants. *Cytokine* 1992, 4:29-35
34. Faherty DA, Claudy V, Plocinski JM, Kaffka K, Kilian P, Thompson RC, Benjamin WR: Failure of IL-1 receptor antagonist and monoclonal anti-IL-1 receptor antibody to inhibit antigen-specific immune responses *in vivo*. *J Immunol* 1992, 148:766-771
35. Schwab JH, Brown RR, Anderle SK, Schlievert PM: Superantigen can reactivate bacterial cell wall-induced arthritis. *J Immunol* 1993, 150:4151-4159
36. Cominelli F, Nast CC, Clark BD, Schindler R, Llerena R, Eysselein VE, Thompson RC, Dinarello CA: Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. *J Clin Invest* 1990, 86:972-980
37. Mulligan MS, Ward PA: Immune complex-induced lung and dermal vascular injury: differing requirements for tumor necrosis factor- α and IL-1. *J Immunol* 1992, 149:331-339
38. Williams RO, Mason LJ, Feldmann M, Maini RN: Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. *Proc Natl Acad Sci USA* 1994, 91:2762-2766
39. Maeda T, Saikawa I, Hotokebushi T, Sugioka Y, Eto M, Murakami Y, Nomoto K: Exacerbation of established collagen-induced arthritis in mice treated with an anti-T cell receptor antibody. *Arthritis Rheum* 1994, 37:406-413
40. Nakajima H, Hiyama Y, Takamori H, Tsukada W: Cell-mediated transfer of collagen-induced arthritis in mice and its application to the analysis of the inhibitory effects of interferon- γ and cyclophosphamide. *Clin Exp Immunol* 1993, 92:328-335
41. Klasen IS, Ladestein RMT, Donselaar IG, van den Berg WB, Tees R, Benner R: Joint inflammation in mice induced by a MT4⁺, LYT-2⁻ T cell clone: characteristics and the induction of flare-up reactions. *J Immunol* 1987, 139:3275-3280
42. Van Lent PLEM, van de Loo FAJ, Holthuysen AEM, van den Bersselaar LAM, Vermeer H, van den Berg WB: Major role for IL-1 but not for TNF in early cartilage damage in immune complex arthritis in mice. *J Rheumatol*, in press
43. Van Lent PLEM, van den Hoek AEM, van den Bersselaar LAM, van de Loo FAJ, Eykholt HE, Brouwer WFM, van de Putte LBA, van den Berg WB: Early cartilage degradation in cationic immune complex arthritis in mice: relative role of interleukin 1, the polymorphonuclear cell (PMN) and PMN elastase. *J Rheumatol* 1994, 21:321-329
44. Martel-Pelletier J, McCollum R, DiBattista J, Faure MP, Chin JA, Fournier S, Sarfati M, Pelletier JP: The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes: identification as the type I receptor and analysis of binding kinetics and biological function. *Arthritis Rheum* 1992, 35:530-540
45. Desmoulins D, Ponteziere C, Agneray J, Ekindjian OG, Cals MJ: Effects of human recombinant IL-1 β on rheumatoid and non rheumatoid human synovial cell growth. *Cell Mol Biol* 1990, 36:309-316
46. Boots AMH, Wimmers-Bertens AJMM, Rijders AWM: Antigen-presenting capacity of rheumatoid synovial fibroblasts. *Immunology* 1994, 82:268-274
47. Huleihel M, Douvdevani A, Segal S, Apte RN: Regulation of interleukin 1 generation in immune-activated fibroblasts. *Eur J Immunol* 1990, 20:731-738
48. Cicuttini FM, Martin M, Boyd AW: Cytokine induction of adhesion molecules on synovial type B cells. *J Rheumatol* 1994, 21:406-412
49. Blue ML, Conrad P, Webb DL, Sarr T, Macaro M: Interacting monocytes and synoviocytes induce adhesion molecules by a cytokine-regulated process. *Lymphokine Cytokine Res* 1993, 12:213-218
50. Shiozawa S, Tanaka Y, Fujita T, Tokuhisa T: Destructive arthritis without lymphocyte infiltration in H2-c-*fos* transgenic mice. *J Immunol* 1992, 148:3100-3104
51. Sack U, Kuhn H, Ermann J, Kinne RW, Voogt S, Jungmichel D, Emmrich F: Synovial tissue implants from patients with rheumatoid arthritis cause cartilage destruction in knee joints of SCID.bg mice. *J Rheumatol* 1994, 21:10-16